Exhibit 6

Human Genetics

Volume 80 No. 2
1988

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Human Genetics

Assignment of the human *hap* retinoic acid receptor RARβ gene to the p24 band of chromosome 3

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Summary. The human *hap* retinoic acid receptor RARβ has been localized by in situ hybridization to the p24 band of chromosome 3.

Introduction

A new gene, named *hap* for hepatoma, has been isolated by characterizing the integration site for hepatitis B virus in a human hepatocellular carcinoma (Dejean et al. 1986). The analysis of the nucleotide sequence of the corresponding cDNA clone clearly identified this gene as a new member of the nuclear receptor multigene family (de Thé et al. 1987). The *hap* product exhibited notably a strong homology with the human retinoic acid receptor (subsequently termed RARα) (de Thé et al. 1987; Giguère et al. 1987; Petkovitch et al. 1987). We have recently demonstrated that *hap* encodes a second retinoic acid receptor, designated RARβ (N. Brand, M. Petkovitch, A. Krust, P. Chambon, H. de Thé, A. Marchio, P. Tiollais, A. Dejean – unpublished work). Using a *hap* genomic single-copy DNA probe, we report here that the RARβ/*hap* gene maps to chromosome 3 p24, close to the region where the thyroid hormone receptor TRβ has been located.

Materials and methods

Chromosome spread preparation

In situ hybridization was carried out on chromosome preparations obtained from human lymphocytes that had been phytohaemagglutinin-stimulated for 72 h. 5-Bromodeoxyuridine was added for the final 7 h of culture (60 μg/ml of medium), to ensure a post-hybridization chromosomal banding of good quality. Slides were treated with RNase and denatured prior to hybridization in 70% (vol/vol) deionized formamide, 2 × SSC (0.3 *M* NaCl, 30 m*M* sodium citrate) at 70°C.

Probe preparation and in situ hybridization

The genomic single-copy DNA probe referred to as RT (Dejean et al. 1986) containing an insert of ≈ 3.500 bp in pBR327 was tritium labelled by nick-translation to a specific activity of 3.9×10^8 dpm/μg. The radiolabelled probe was hybridized to metaphase spreads at a final concentration of 500 ng/ml of hybridization solution as previously described (Mattei et al. 1985).

Autoradiography, staining and banding

After coating with nuclear track emulsion (Kodak NTB2), the slides were exposed for 8 days at 4°C, then developed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with buffered giemsa solution and metaphases protographed. R-banding was then performed by the fluorochrome–photolysis–giemsa (FPG) method and metaphases re-photographed before analysis.

Results and discussion

In the 100 metaphases examined after in situ hybridization, there were 191 silver grains associated with chromosomes and 57 of these (29.8%) were located on chromosome 3 (Fig. 1). The distribution of grains on this chromosome was not random: 75% of them mapped to the p22→p24 region of the chromosome 3 short arm with a maximum in the 3p24 band (Fig. 2). These data strongly suggest that the retinoic acid receptor RARβ is located on the p24 band of chromosome 3. The retinoic acid receptor RARα has been recently located to the q21 band of chromosome 17 (Mattei et al. 1988). It is interesting to note that the RARα and RARβ are more homologous to the two closely related thyroid hormone receptors TRα and TRβ than to any other members of the nuclear receptor family (de Thé et al. 1987; Giguère et al. 1987; Petkovitch et al. 1987). The thyroid hormone receptor TRβ maps to chromosome 3p21.33–22 (Drabkin et al. 1987), the thyroid hormone receptor TRα (also termed *erbA1*) maps most probably to chromosome 17q11.2→q12 (Robertson 1987) while two other *c-erbA*-related genes, *erbA2* and *erbA2*-like, have been mapped respectively to chromosome 17q21.3 and 17q25 (Gosden et al. 1986).

Analysis of the gene family encoding the nuclear receptors has shown that they can be roughly divided into two groups: the steroid receptors, which have different chromosomal localizations (Mattei et al. 1988), and the non-steroid receptors, which appear to be located on either chromosome 3 or 17. This observation suggests that the genes encoding the

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Fig. 1a, b. Three partial human metaphases showing the specific site of hybridization to chromosome 3. **a** *Arrowheads* indicate silver grains on Giemsa-stained chromosomes after autoradiography. **b** The same chromosomes with silver grains were subsequently identified by R-banding (FPG technique)

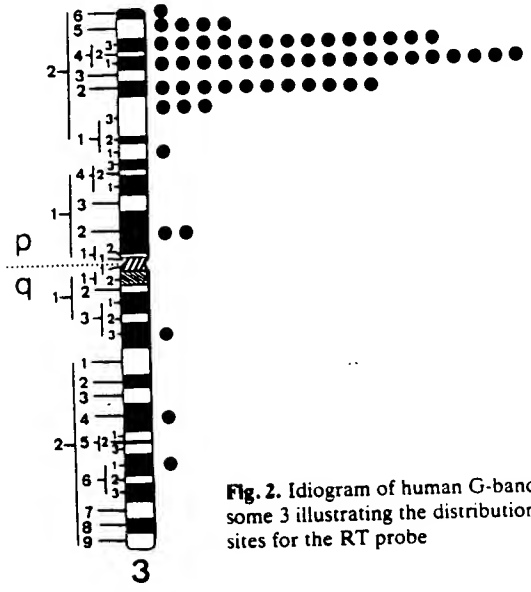

Fig. 2. Idiogram of human G-banded chromosome 3 illustrating the distribution of labeled sites for the RT probe

thyroid hormone and retinoic acid receptors have evolved by duplication of an ancestral gene, which itself diverged earlier in evolution from the steroid hormone receptor progenitor.

Acknowledgements. We thank Edith Passage for efficient technical assistance. This work was supported by NIH grant CA-97300 and the Fondation pour la Recherche Médicale.

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Received April 7, 1988